Calcium Alginate Bead Based Scaffold For Drug Delivery And Tissue Engineering

A THESIS SUBMITTED FOR PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF

BACHELOR OF TECHNOLOGY

IN

BIOTECHNOLOGY

By:

PRAJNA KABIRAJ

(Roll no. 110BT0447)

Under the guidance of:

Prof. INDRANIL BANERJEE

Department Of Biotechnology And Medical Engineering

National Institute Of Technology Rourkela

2014

NATIONAL INSTITUTE OF TECHNOLOGY

ROURKELA

DEPARTMENT OF BIOTECHNOLOGY AND MEDICAL ENGINEERING

C E R T I F I C A T E

This is to certify that the thesis entitled *"Calcium Alginate Bead Based Scaffold For Drug Delivery And Tissue Engineering"* submitted by Ms. Prajna Kabiraj [Roll No. 110BT0447] in partial fulfilment of the requirements for the award of the degree of Bachelor of Technology in Biotechnology at National Institute of Technology, Rourkela is an authentic work carried out by her under my guidance.

To the best of my knowledge the matter embodied in the thesis has not been submitted to any other University/Institute for the award of any degree or diploma.

Date: 10th May 2014.

Dr. Indranil Banerjee

Department of Biotechnology and Medical Engineering

National Institute of Technology

Rourkela-769008

ACKNOWLEDGEMENT

"KNOWLEDGE IS INCOMPLETE WITHOUT MOTIVATION AND DIRECTION"

I would really like to take this opportunity to thank my project guide, **Prof. Indranil Banerjee**, Department of Biotechnology and Medical Engineering, NIT Rourkela, for believing in me and allowing me to work on this project and motivating me throughout the time.

I am sincerely thankful to **Prof. K. Pramanik**, **Prof. K. Pal** and **Prof. S.S.Ray,** Dept. of Biotechnology and Medical Engineering, NIT, Rourkela, for providing the necessary facilities for this work. I gratefully extend my sincere thanks to all faculties and to all teaching and non-teaching staff members of Department of Biotechnology & Medical Engineering, National Institute of Technology Rourkela, Orissa

My special thanks to research scholar **Mr. Tarun**. I also owe a debt of gratitude to **Mr. Senthilguru, Mr. Satish, Mr. Vinay, Mr. Prerak, Ms. Shrutija, Ms. Somya** and **Mr. Goutham** for all the help and support I got from them. This final year project has brought the best in me and I have given everything to be able to live up to the expectations of all.

I would like to express my heartily thanks to my friends Shambhavi Singh, Satabdee Mohapatra, Dattaram Sugave, labmates and others in the department for their help and support.

Finally, I would like to express my heartfelt thanks to my parents for their blessings, support and constant encouragement and very special thanks to God for showering the blessings on me.

Prajna Kabiraj

B.Tech (Biotechnology Engineering)

National Institute of Technology, Rourkela

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

ABSTRACT

Implants are medical devices that are fabricated in order to substitute, support or improve the biological structures. Generally, such implants are composed of biocompatible materials (synthetic polymers and natural biopolymers, metals, metal alloys) depending upon their applications. In this regard, we fabricated a novel three dimensional calcium alginate bead based scaffold implant for drug delivery and tissue engineering applications. The scaffold was designed by placing the alginate beads in layer by layer arrangement allowing hexagonal closed packing. The designed scaffold was analyzed for its physico-chemical characteristics using SEM, FTIR, swelling behavior and drug release kinetics. Further, biological characterizations such as biocompatibility and antibacterial activity of the scaffolds were also carried out. The fabricated scaffold had dense and compact packing of the calcium alginate beads resulting in better mechanical strength. Analysis of release kinetics of model drug (Rhodamine) showed that the release rate of the drug was dependent on the number of layers stacked over each other. The scaffold implant was found to be biocompatible. Thus, we decipher that the scaffold implant could be used for drug delivery and tissue engineering applications.

KEYWORDS: Sodium alginate, Rhodamine dye, scaffold, implant, biocompatibility, alginate beads.

CHAPTER 1

INTRODUCTION & LITERATURE REVIEW

1.1 Introduction:

In recent years bioactive implants have started gaining attention. Biologically active implants are the system when placed in vivo interact with the system in a positive way that helps in restoring or repairing the damaged or traumatized tissue. Usually there are two types of biologically active implants; the first one is "Implants made up of metal or ceramics coated with bioactive molecules" (i.e. cell adhesion proteins, peptides, growth factors, drugs)[1]. The second type is "Tissue inductive scaffolds"[2].

Recently people have come up with a new hybrid system that possess the virtue of both the implants mentioned above. Such implants are prepared by loading the bioactive compounds in tissue engineered scaffold[3]. Beauty of this system is that it can contribute multiple therapeutic benefits simultaneously or following a desired spatiotemporal sequence. There are a number of such implants now in clinical practice including microsphere impregnated porous scaffold, microsphere impregnated hydrogel, nanoparticle loaded film, and different beads based systems.

Examples of the implants now in clinical use:

A porous collagen scaffold when impregnated with ciprofloxacin loaded gelatin microspheres, it is capable of delivering the drug at the wound site in a very controlled manner. Water-in-oil emulsion technique was used to prepare the ciprofloxacin loaded gelatin microspheres and were then impregnated in the collagen sponge[4]. The pore size of collagen sponge is in the range of 500 - 700 μm, which can encapsulate the gelatin microspheres. At the wound location the gelatin microspheres would degrade offering drug release at the wound surface and the collagen sponge acts as reservoir for gelatin microspheres; it also supports skin redevelopment.

In another research gelatin microspheres were prepared containing basic fibroblast growth factor (bFGF) for its controlled release. When preadipocytes were implanted simultaneously with gelatin microspheres, they were able to induce adipose tissue development at the implanted site. After isolating preadipocytes from human adipose tissue, they were suspended with the gelatin microspheres which contained bFGF and were merged into a collagen sponge of cell scaffold[5].

A novel and smart drug delivery system was developed comprising hydroxyl functionalized glycerol poly (ecaprolactone) (PGCL)-based microspheres and poly (N-isopropylacrylamide) (PNIPAAm) hydrogel. Several quantities PGCL based microspheres were introduced into poly (N-isopropylacrylamide) (PNIPAAm) hydrogel[6]; which is temperature sensitive, to produce the novel drug delivery systems.

Nanoparticle loaded thin films is used in active research's for the longest period of time among these technologies. The unusual properties of metal nanoparticles are originated from quantum confinement effects and their large specific surface area. The applications of nanoparticles distributed in an organic matrix are: catalysis, magnetics, and photonics[7, 8]. The nanoparticle loaded polymer coating on colloidal particles is desired as colloidal particles can offer more surface area which is advantageous for a wide range of applications, such as catalysis and sensor applications[9].

Among these systems; the beads based system shows real promises because of its easy processing, small size, more volume, and more surface area to volume ratio. Smaller beads also offers advantages like; such as better conveyance of nutrients and oxygen, better mechanical strength, improved dispersion, easier grafting, and possible access to new implantation locations[10].

The systems are already in use for cartilage tissue engineering[11], dual drug delivery (as in scaffold)[12], controlled release of multiple growth factors[13], protein delivery[14], controlled release of interleukin-2 for tumor immunotherapy[15], biodegradable cell carrier which helps in further tissue maturation[16].

1.2 LITERATURE REVIEW:

1.2.1 Bioactive Implants:

Bioactive glass, bioactive calcium phosphate ceramics, bioactive glass ceramics, bioactive composites and coatings are the biomaterials that can bind to living tissue[17]. The biomechanical and biochemical compatibility of the materials can be optimized using two methods: (a) structural tailoring of bioactive materials[18] and (b) molecular tailoring of surface chemistry[19]. There are two types of bioactivities, osteoconductive and osteoproductive bioactivity, due to different mechanism and rates of implant-tissue interactions. The prostheses made up of bio-inert materials have the survivability half-life period of approximately 15 years (clinical applications are taken into account)[18]. Device lifetime can be improved with the help of bioactive materials but it has its limitations. Instead of this method use of hierarchical bioactive scaffolds or resorbable bioactive particulates or porous networks can be done to engineer in vitro living cellular constructs for transplantation and to activate in vivo the mechanisms of tissue regeneration.

1.2.2 Implantable beads:

The major disadvantages of parental antibiotic therapy for soft tissue infections, acute bone infections and osteomyelitis can be associated with ototoxic, nephrotoxic, can result high concentrations of serum and can also be allergic complications[20]. Due to these drawbacks antibiotic loaded biodegradable implants are in more use for bone infection treatment.

Among a number of implants, the biocompatible and bioresorbable drug loaded beads are more in use for the treatment of tissue disorder. The drugs that can be loaded for various treatment can be; antibiotics for treatment of bone infection, antineoplastic substances for treatment of cancer and analgesics for relieve from pain etc.[21]. For insertion and better handling the beads can be attached to any bioresorbable junction. A good example of the bioresorbable material that can be

used for such type of applications is calcium sulfate, as it is biocompatible and has radiopaque property. Calcium sulfate beads can be used as bioresorbable void filler for bone or X-ray markers[22].

One more use of implantable beads is that; wen the beads are made up of agarose and collagen or coated with agarose have been incorporated within cell samples. The cells produced biological products which are diffusible[23]. These beads can be arranged into an implant to control the recipient's immune response. The beads can also be used in vitro to boost or suppress the growth of specific cells or to produce desired product from culture.

Applications and Scopes:

a) Beads that can be used as implant if contain agarose and collagen and if are coated with agarose if introduced into cells, they yield biological products which are diffusible. These beads can also be useful to control receiver's immune reaction. The beads can also be used to enhance or suppress the growth of a particular cell in vitro and to produce the desirable result during cell culture. An example of such use was proved when the proliferation of the renal cancer cells was restricted by entrapping in the beads made up of material that suppresses cancer cell growth [24].
b) The beads made up of polymethylmethacrylate and incorporated with gentamicin had shown that the release rate can be very slow and the release time can be extended up to a time interval of several months and the concentration high enough to control the pathogens. The therapeutic efficiency of such drugs had been experimented on the model of osteomyelitis in the femur of hound. The tolerance was found to be sufficiently good in case of both tissue cultures and animal models. Gentamicin-PMMA beads insertion can be a valuable modern technology for local antibiotic therapy [25].

c) An example of the above described method was when the radius of 77 rabbits were introduced with osteomyelitis and after 4 weeks the wound were debrided and the rabbits were treated with fatty acid dimersebacic acid beads loaded with 10-20% gentamicin sulfate or placebo beads and intramuscular gentamicin sulfate or only placebo beads or debridement only. Then again after 4 weeks the extinction of infection was determined by histological analysis and culturing. The animals treated with beads containing 20% gentamicin, among them 93% of animals were found to be osteomyelitis free, among the animals treated with beads containing 10% gentamicin, 67% were found osteomyelitis free, in 25% of those cured with intramuscular gentamicin and placebo beads, in 12.5% of those treated with debridement only, and in 7% of those treated with placebo beads alone [26] .

d) Another method among this techniques is the preparation of PLA biodegradable beads which can be impregnated with an ionic amino-glycoside and gentamycin. This process involves hydrophobic pairing of ions for solubilizing of gentamycin in a solvent which is compatible with poly-L-lactide (PLA), then precipitation with a compressed antisolvent (supercritical CO_2) was done. The precipitate was found to be a homogeneous distribution of the ion combined drug in PLA microsphere. The bead sequences show no major change in release kinetics when sterilized with a H_2O_2 plasma. The gentamycin release kinetics from the PLA beads steady with the mechanism of matrix organized diffusion. Whereas the release rate of non-biodegradable PMMA beads were same as gentamycin initially and then ceases after 8-9 weeks while the release in PLA beads continues for 4 months[27].

Advantages:

The beads based system shows real promises because of; a) easy processing, b) small size, c) high drug loading capacity, and d) more surface area to volume ratio. Smaller beads also offer;

like i) better transportation of nutrients and oxygen, ii) better dispersion, iii) better mechanical strength, iv)easier implantation, and v) potential access to new implantation sites.

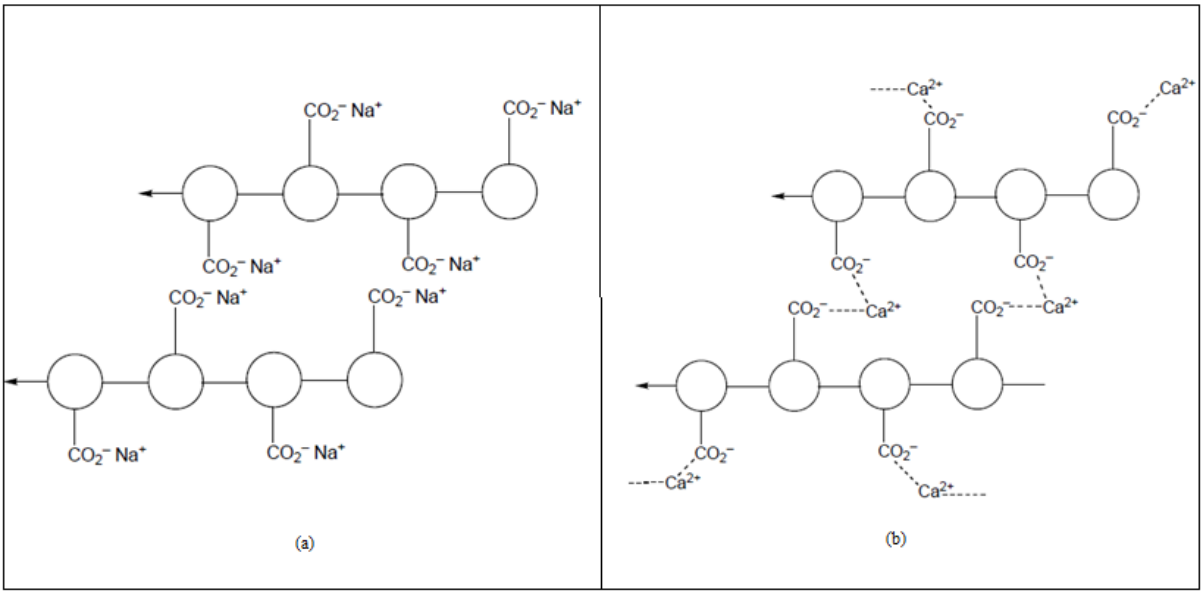
1.2.3. Calcium Alginate Beads:

1.2.3.1. Sodium Alginate: Alginates are the chemicals extracted from the brown seaweeds and are available in ammonium, sodium and potassium derivatives. Alginates can be dissolved in both cold and hot water. It can also harden and bind. In presence of any specific acid or calcium alginates can form tough gels. Sodium alginate is the sodium salt of alginic acid. It is in form of gum when removed from the cell walls of brown seaweed and can be used in food industries to increase viscosity. It can also be used as an emulsifier. It is also used as a component in indigestion tablets. It does not has any distinguishable flavor. It is a cold gelling agent which needs no external heat to gel, it dilutes while cold with strong agitation. However for the gel formation of this compound calcium compound is needed to be present. Usually calcium chloride is used to make caviar and spheres of alginate. Heat is not required to produce spherification. Sodium alginate can also be used for the formation of foams[28].

1.2.3.2. Calcium Alginate: It is a gelatinous, water-insoluble, and cream colored substance that can be formed by adding aqueous $CaCl_2$ with aqueous Na. alginate. Calcium alginate is also in use for forming artificial seeds in plant tissue culture and entrapment of enzymes. The optimization and improvement of calcium alginate for possible use in endovascular occlusion was examined by analyzing it's in vitro and in vivo biocompatibility and mechanical stability. The compressive rheology, resistance, and polymer profit of reacted alginate along with the polymer viscosity of unreacted alginate, were evaluated. Biocompatibility of calcium alginate had been established by

injecting it into the kidney capsule of mice. The reactivity of alginates with various compounds and at different levels of purity were associated histologically and visually. Consequences suggested that calcium alginate is a mechanically stable and biocompatible gel for endovascular uses. Refined alginates showed compressive strength of about 22 kPa and above at a level of 40% compression, with no major change in elasticity. Strength of Cleansed alginate was considerably greater than the crude alginates. The tissue reaction of purified alginates also presented considerably lower than that of crude alginates. Among all the alginates verified, cleansed high guluronic acid alginates showed ideal polymer return and strength, decreased viscosity, and increased biocompatibility. Medical embolization therapies can be developed with the progress of biocompatible and stable polymers like calcium alginate. Possible practices of upgraded endovascular polymers comprise vascular hemorrhaging, blood flow to tumors, aneurysms and treatment arteriovenous malformations i.e. AVMs [29].

1.2.3.3. Crosslinking Mechanism: The polymer of sodium alginate can be signified as it is represented in fig.1 (a).



(Fig. 1)

Once sodium alginate has been put in a solution that contains calcium ions, then the calcium ions will replace the sodium ions in the polymer. Each calcium ion can confer maximum two of the polymer threads [30]. This mechanism is known as cross-linking and can be symbolized as given in fig.1 (b).

This crosslinking machinery is essential for converting sodium alginate to calcium alginate; a gelatinous substance which can be water-insoluble and is beneficial for a number of experiments.

1.2.3.4. Bead Formation: Alginate can form gels with a wide range of divalent cations. By the method of emulsification coupled with internal gelation technique spherical calcium alginate beads with uniform and small size were effectively prepared [31]. We can control the size and the size distribution of the beads by regulating process parameters. Additional research on preparation of alginate chitosan microspheres with the help of this technology for controlled release and loading of drugs in is still in progression.

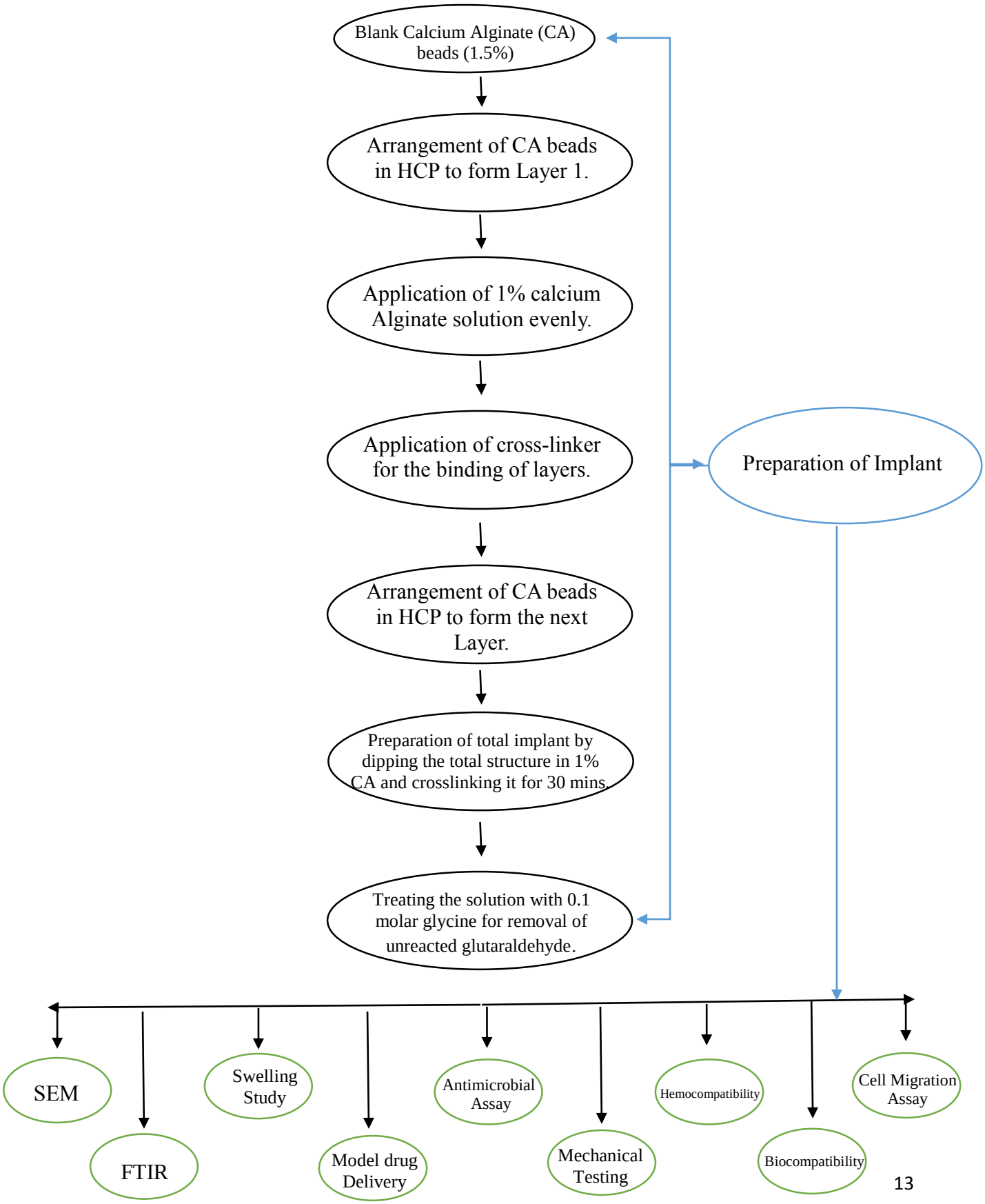
CHAPTER 2

OBJECTIVES AND WORK PLAN

2.1. OBJECTIVES:

- Preparation of calcium alginate bead based scaffold
- Physico-chemical characterization of scaffold
- Potential application of the scaffold in drug delivery and tissue engineering

2.2. **WORKPLAN:**


Blank Calcium Alginate (CA) beads (1.5%)
Arrangement of CA beads in HCP to form Layer 1.
Application of 1% calcium Alginate solution evenly.
Application of cross-linker for the binding of layers.
Arrangement of CA beads in HCP to form the next Layer.
Preparation of total implant by dipping the total structure in 1% CA and crosslinking it for 30 mins.
Treating the solution with 0.1 molar glycine for removal of unreacted glutaraldehyde.
Preparation of Implant
SEM
FTIR
Swelling Study
Model drug Delivery
Antimicrobial Assay
Mechanical Testing
Hemocompatibility
Biocompatibility
Cell Migration Assay

CHAPTER 3

MATERIALS AND METHODS

3.1. Materials:

Sodium Alginate (SA) was bought from SDFCL, Mumbai, India. Calcium Chloride ($CaCl_2$, fused) was purchased from MERCK, Mumbai, India. The glutaraldehyde (25% aqueous solution) was procured from LOBA Chemie, Mumbai, India. Carboxymethly Cellulose Sodium (CMC) salt, Dulbecco's Modified Eagle Media (DMEM), Dulbecco's Phosphate Buffer Saline (DPBS), Trypsin EDTA Solution, Fetal Bovine Serum, Antibiotic-Antimycotic Solution, MTT Assay Kit and Nutrient Broth were purchased from Himedia, Mumbai, India. The MG63 cell line procured from NCCS, Pune.

3.2 Methods:

3.2.1. Preparation of Beads based implants:

Calcium alginate beads were made by ionic gelation method by using 1.5% (w/v) sodium alginate and crosslinking solution containing calcium chloride (2% w/v) & glutaraldehyde (0.25% v/v). [redacted] (protected for publication) The implant was then dipped in 0.1 molar glycine solution for another 60 minutes for neutralization of the uncrosslinked glutaraldehyde as it is toxic for cells [31].

3.2.2. Scanning Electron Microscopy:

The morphology of the dried implant was observed by field emission scanning electron microscope (FE SEM) (Nova NanoSEM 450). Prior to visualization, the samples were sputter coated with gold using sputter coater (Quorum Technologies, Q150RES). The scanning was performed at 5KV [32].

3.2.3. Fourier Transform Infrared Spectroscopy:

The Fourier transform infrared spectroscopy (FTIR) of dried implant and pure sodium alginate powder was done using FTIR spectrophotometer AKTR (Shimadzu/IR prestige 21) using KBr pellet in the scanning range of 4000 – 400 cm^{-1}[33].

3.2.4. Swelling Analysis:

The swelling analysis of the dried and wet implants was done in Phosphate Buffer Saline (PBS, pH 7.4) and culture media with (% calcium content) at 37^{o}C. For this, the implants were weighed and dipped in 25ml PBS [32]. At definite time, the implants were taken out, carefully blotted with filter paper and increase in weight of implants was measured using formula:

$$\textbf{Swelling Ratio (SW)} = (W_t - W_0) / W_0$$

Where, W_0 and W_t represent initial weight of implant and weight of implant at time‘t’ respectively.

Moreover the change in the implant dimensions i.e., length, breadth and height were measured using a Vernier caliper (Fisher Scientific) to the nearest of 0.001mm. The measurements were taken at least at 3 random position for each dimension and the average value was reported.

3.2.5. Fabrication of Model drug loaded implant & Study of drug release:

Rhodamine was used as a model drug for the study of release kinetics. [redacted] (protected for publication) The release of dye was observed for both three layer and five layer implant structures.

3.2.6. Study of drug release from the implant:

Metronidazole loaded implant, fabricated in a similar way as discussed in the section [2.2.5] was evaluated for its antibacterial activity against *Escherichia coli* using agar diffusion method. For this, UV sterilized fresh implant was placed in a 90mm petri dish. Thereafter, the petri was filled with nutrient agar and allowed to solidify. Further, 50µl of *E. coli* culture was spread onto the plate and was kept for incubation at 37^{o}C for overnight. The extent of release of drug was determined by the zone of inhibition [34].

3.2.7. Mechanical Testing of implant:

Prepared substrates were characterized for compression and stress relaxation profile using TA.XT2i Texture Analyzer (Stable Micro Systems Ltd, Surrey, UK). Substrate stiffness was calculated in terms of the modulus of deformability using compression data.

Table. 1: The parameters used while testing mechanical properties

Type of study	Type of fixture	Pre-test speed	Test speed	Post-test speed	Mode of study
Stress relaxation	3mm probe	1mm/sec	1mm/sec	1mm/sec	Auto (Force) 5g,3mm
Compression	30mm probe	1mm/sec	1mm/sec	1mm/sec	Auto (Force) 5g,30mm

3.2.8. Hemocompatibility:

The hemocompatibility of the CA implant was analyzed using fresh goat blood. In brief, the whole goat blood was diluted in 1:1 ratio using normal saline (0.89% NaCl). Thereafter, 0.5ml of leachant from CA implant was added to diluted blood sample. The positive and negative controls were prepared by placing 0.5ml of 0.1N HCl and normal saline respectively instead of test samples. The samples were then centrifuged at 1000rpm and the percentage hemolysis was analyzed by measuring the absorbance at 545nm using UV-visible spectrophotometer. The leachant preparation was carried out by placing implant (1cm x 1cm x 1cm) in PBS (pH 7.4) at 37^{o}C overnight, followed by centrifugation at 3000rpm and the supernatant was used for the analysis [35].

3.2.9. Biocompatibility:

The biocompatibility of fabricated implant was analyzed against HaCaT cell line (human keratinocytes) using the leachant. In brief, the cell line was maintained in DMEM with 10% FBS in a humidified (95%), CO_2 incubator (5%) at 37^{o}C. Cells were harvested, evaluated for their viability and seeded in a 96 well plate at a concentration of 1x10^{5}cells/ml. Further, 20 µl of filter sterilized leachant was added in each well. The leachant was prepared by placing

implant in PBS (pH 7.4) at 37^{o}C overnight. The sample was then centrifuged at 3000rpm and supernatant was used for the analysis. The culture plate was then incubated for next 24 hours and cytotoxicity of leachant was analyzed using MTT Assay. The same protocol was followed to optimize the neutralization time of glutaraldehyde. For this, the implants were neutralized for various time intervals (0, 2, 4, 6, 24 hours) using 0.1M glycine prior to leachant preparation and the cell viability was evaluated for each [36].

3.2.10. Cell Migration Assay:

The cell migration into the fabricated implant was evaluated using MG63 cell line (human osteosarcoma). For this, the implants were fabricated, crosslinked, neutralized, equilibrated with DMEM media for next 24 hours and seeded with MG63 cells under sterile conditions as explained in section [2.2.1]. The MG63 cells were maintained in DMEM with 10% FBS in a humidified (95%), CO_2 incubator (5%) at 37^{o}C. Cells were harvested, evaluated for their viability and seeded onto the implant a concentration of $1x10^{6}$ cells/ml. At definite time interval, the cell viability was evaluated using MTT assay. [36]

CHAPTER 4

RESULT & DISCUSSION

4.1. RESULTS:

4.1.1. Fabrication of CA bead based implant:

Alginate beads have been prepared by a number of research groups for the delivery of drugs, proteins and peptides. However, calcium alginate beads based implants has not been in a wide use. [redacted] (protected for publication) The structure was found to be stable and handling as compared to beads became easier. By placing the structures at 37 ^{0}C for 24 hours, it dried completely. Upon drying the structures shrunk to a very compact form[12].

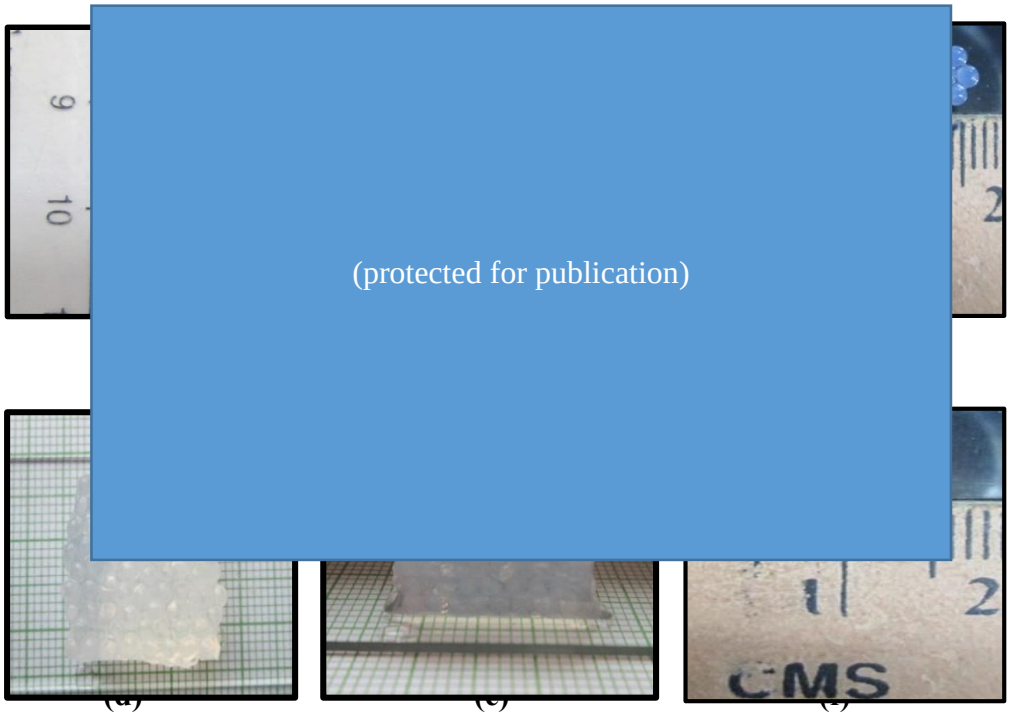


Fig. 1: (a) fabrication of beads and scaling, (b) Hexagonal close packing of beads, (c) Hexagonal close packing of normal beads and Rhodamine loaded beads, (d), (e) implant fabrication and scaling, (f) Scaling of dried implants

4.1.2. Scanning Electron Microscopy:

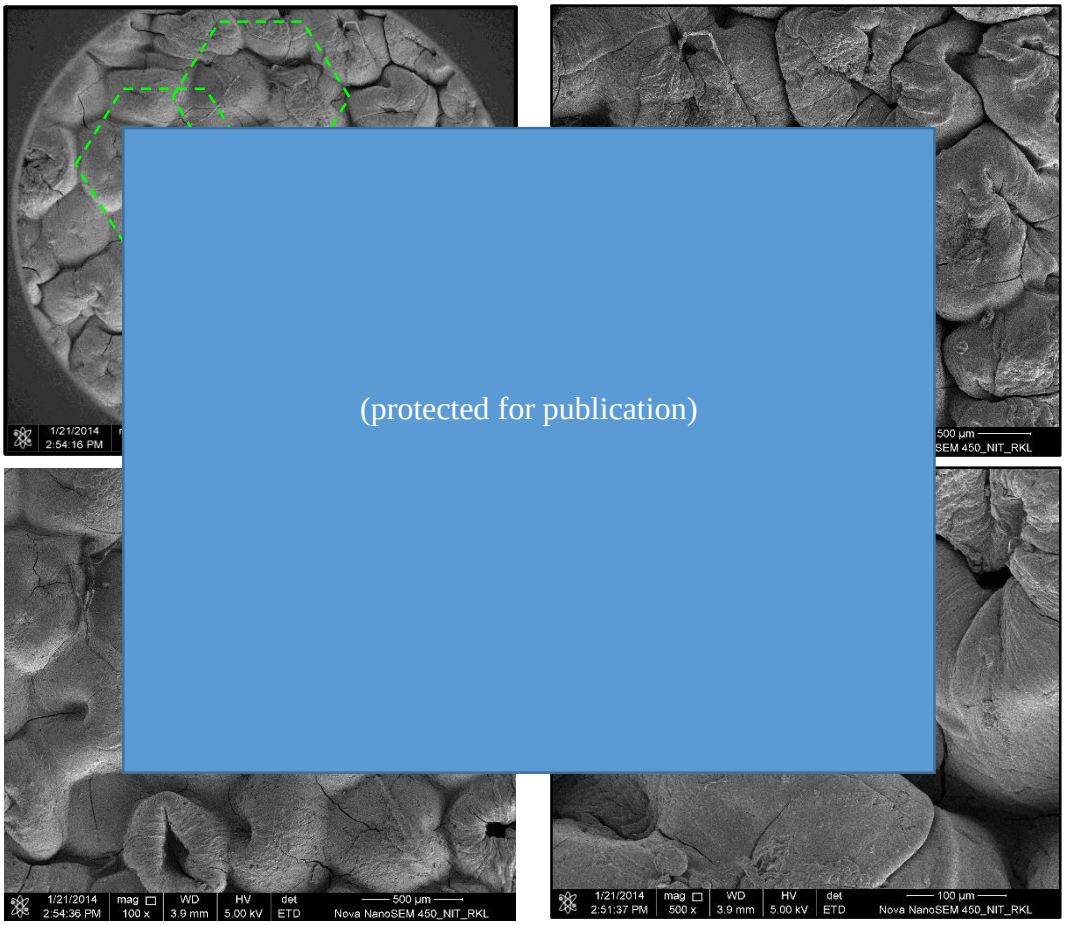


Fig. 2: Surface morphology of CA bead implant

Analysis of surface morphology of dried structures by Scanning Electron Microscopy showed that [redacted] (protected for publication) The beads in the center of the structure are intact but at the edges they were almost squeezed have formed a wrinkled surface [32].

4.1.3. Fourier Transform Infrared Spectroscopy:

FTIR spectra analysis of pure SA and the formulated implant demonstrated the presence of characteristic peaks at 1620cm^{-1}, 1420cm^{-1} and 1306cm^{-1} corresponding to symmetric and

asymmetric vibrations of carboxylate ions in SA. Broad peak near 3400cm^{-1} corresponds to OH stretching mode. The peaks present in 1200-1000 cm^{-1} region represents the absorption due to sugar ring. It is important to mention that the similar peaks were observed in case of calcium alginate implant. However the percentage absorption of the peaks tends to increase. This may be a result of polymeric chains of alginate by calcium and glutaraldehyde [33].

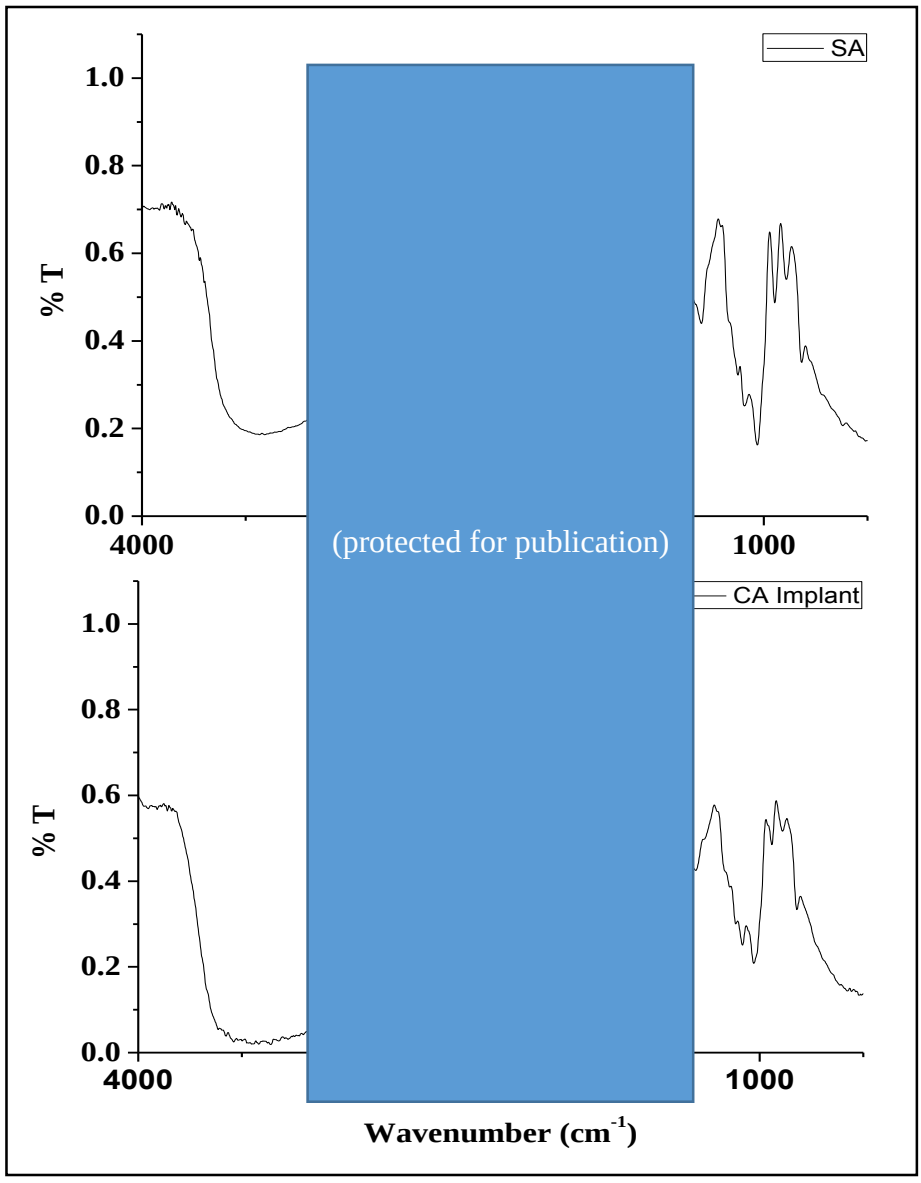
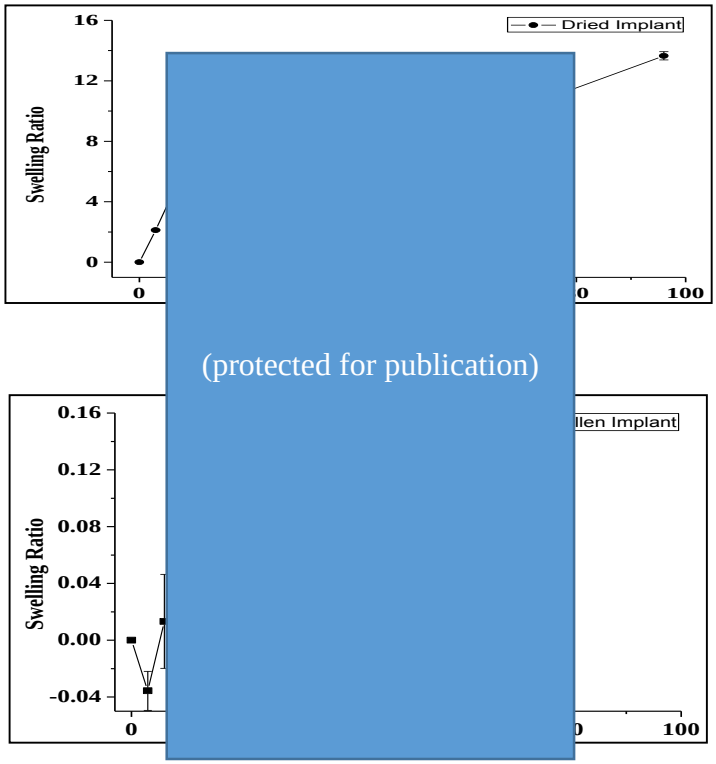


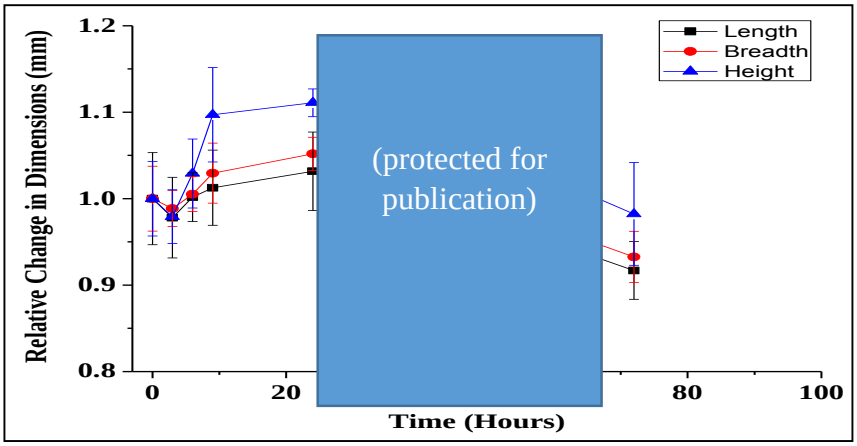
Fig. 3: FTIR Analysis

4.1.4. Swelling Analysis:

The swelling analysis of dried and swollen implant was carried out in PBS at the physiological pH 7.4. The swollen structure did not show much increase in the swelling ratio. [redacted] (protected for publication). Thereafter the swelling ratio increased with time till 48 hours of study and then decreased due to disintegration of the structure. [redacted] (protected for publication). Dried structure demonstrated fast swelling for initial few hours of analysis. Thereafter, swelling of structure occurred at a steady rate. During swelling, the dried implant retained its structural integrity and disintegrated after a span of 96 hours [32].



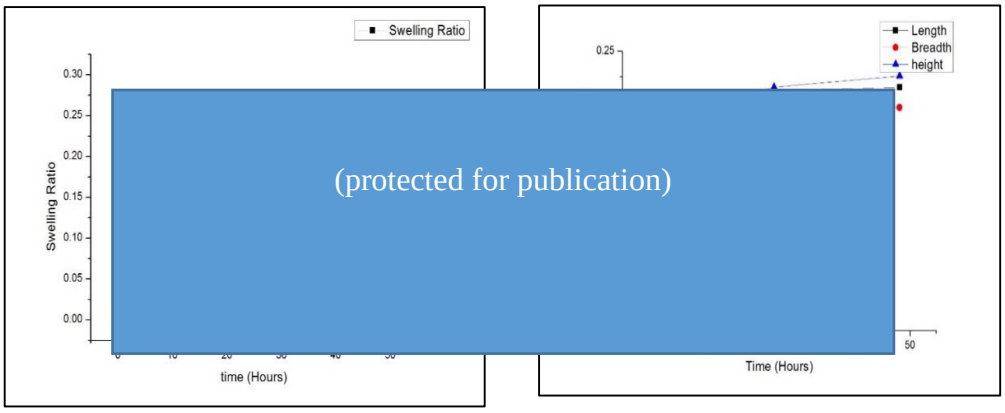
(b)



(c)

Fig. 4. (a) Swelling ratio of dried implant, (b) Swelling ratio of swollen implant (c) Relative change in dimensions for swollen implant

The swelling analysis of swollen implant was also carried out in culture media at the physiological pH 7.4. [redacted] (protected for publication) Thereafter the swelling ratio increased with time; in the beginning it increased in a very high ratio thereafter in a very uniform way. During swelling, the implant retained its structural integrity and disintegrated after a span of 48 hours [32].



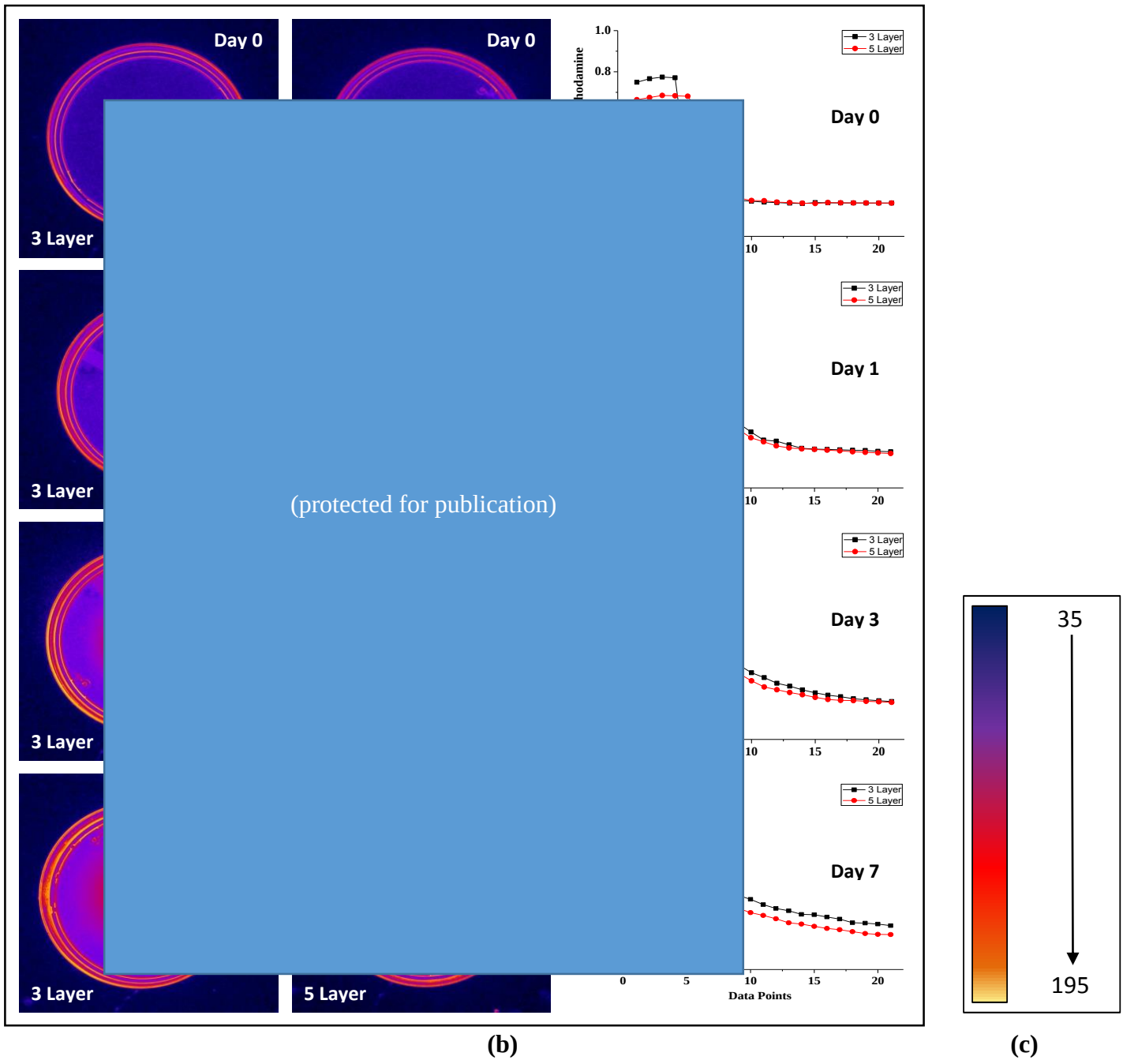
(a) **(b)**

Fig. 5: (a) Swelling ratio for swollen implant, (b) Relative change in dimensions of swollen implant

4.1.5. Fabrication of Model drug loaded implant & its release study:

In case of scaffolds, the homogeneous distribution of drugs is very difficult to attain. The scaffolds which are designed by freeze drying or salt leeching method have a very random non-spatial distribution of drugs. In a definite time interval, implant having more numbers of bead layers showed greater interlayer release of the drug before it is dispersed into the surroundings, hence giving a lag time in its release [35].

(a)

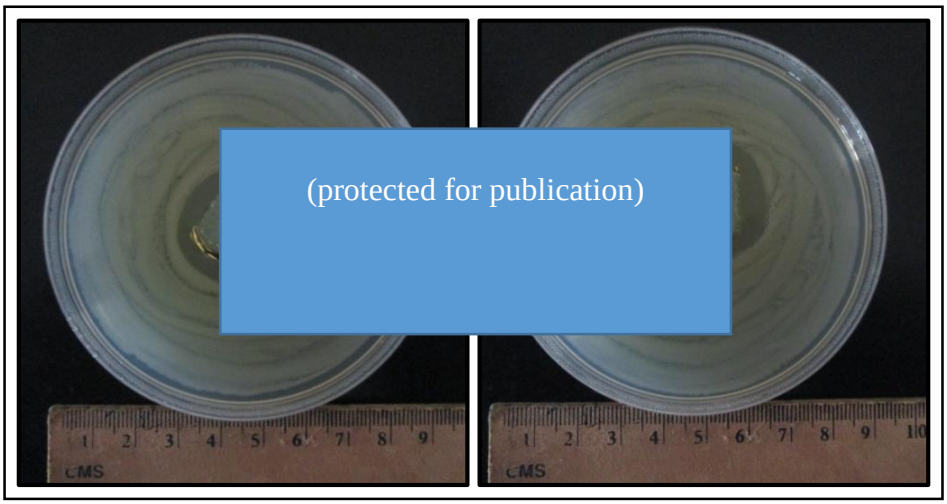


(b) **(c)**

Fig. 6: (a) Analysis of Rhodamine diffusion from bead implants to agarose after 7 days, (b) Analysis of intensity profile from center towards periphery as a function of distance, (c) Intensity scale for fig. 6(b)

4.1.6. Drug loaded implant & its release study:

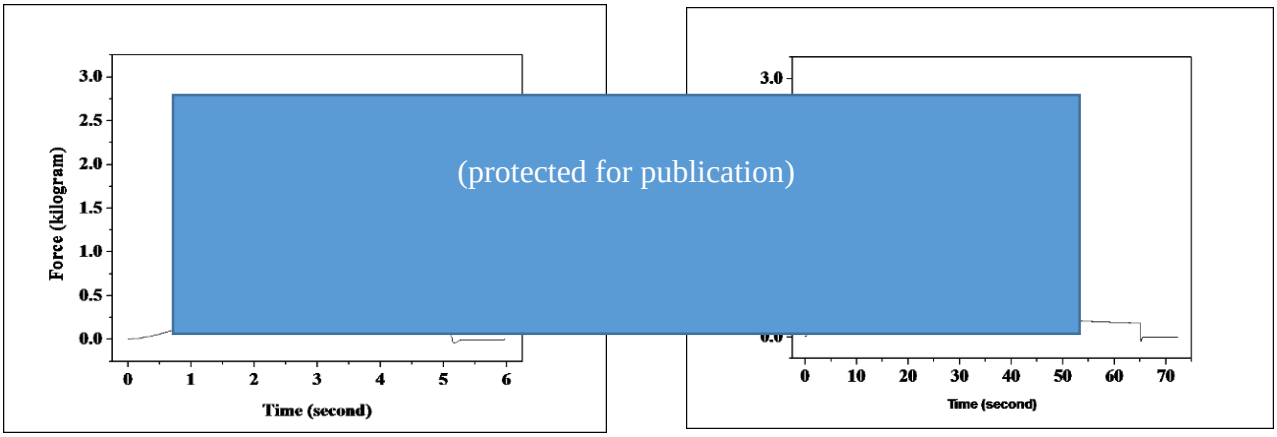
When the anti-bacterial analysis was completed on the CA implants each gave a clear zone of inhibition. The drug used for this study was metronidazole. The zone of inhibition around the metronidazole implant was due to the release of the same by diffusion. [redacted] (protected for publication)



(a) **(b)**

Fig. 7: Zone of inhibition for metronidazole loaded implants after 24hrs

4.1.7. Mechanical Testing of implants:



(a) **(b)**

Fig. 8: (a) Compression Analysis, (b) Stress relaxation Analysis

Mechanical properties are important for handling and processing of the implants. The mechanical properties of the implants were evaluated on the basis of 'compression' and 'stress relaxation'. [redacted]

(protected for publication).

4.1.8. Hemocompatibility:

To determine if a sample is suitable for in vivo applications the interaction of the polymeric origination with blood is necessary parameter. Usually the hemolysis percentage caused by the polymer is the primary criteria to measure its hemocompatibility. The result found from our experiment confirmed that the CA implants are greatly hemocompatible with a percentage hemolysis of about 3% [35].

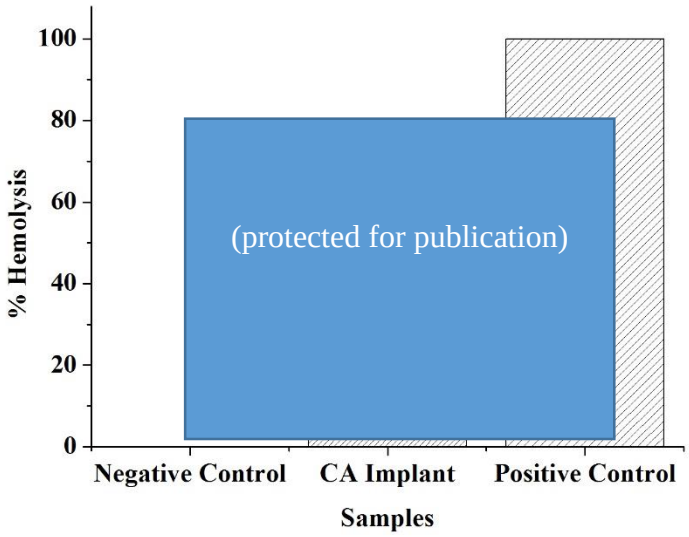


Fig. 9: Hemocompatibility Analysis of CA implants

4.1.9. Biocompatibility:

When the integration of the implants were considered with the tissue, it is important to estimate the interactions of the implant with the cells. If an implant supports the cells while adhering and proliferating and does not cause any cytotoxic effect then it can be considered as biocompatible. [redacted].(protected for publication) Though, due to the presence of unbound glutaraldehyde restricts the compatibility of the implant because of its cytotoxic properties. Therefore, it is extremely vital to counteract the unbound glutaraldehyde by 0.1M glycine; still the time of treatment needs to be optimized [36].

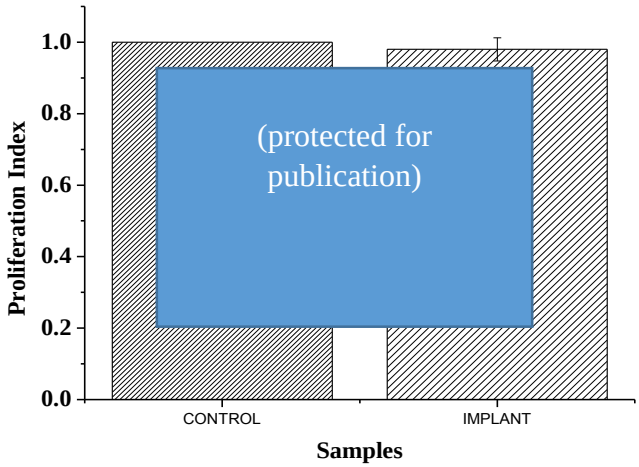


Fig. 10: Biocompatibility analysis of CA bead based implants

4.1.9. Cell Migration Assay:

A cellular scaffold should contain good population of cells required. The present cell seeding method failed to confirm such standardized seeding; particularly for scale of higher 3D volume. In these type of cases cell migration from external environment to inside of scaffold can only guarantee standardized cell populations. This is the reason for fabricating scaffolds having significant porosity. This is one of the major reason of making scaffold having significant pore size and porosity. From the point of view of tissue engineering a microarchitecture like this which helps the migration of the cells inside the scaffold is advantageous. [redacted] (protected for publication).

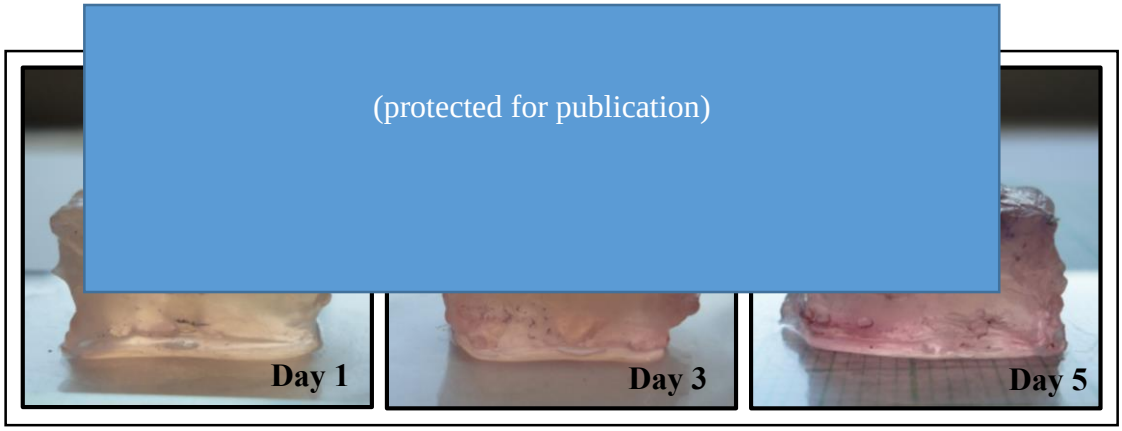


Fig. 11: Cell migration within the bead based scaffold at day 1, day 3 and day 5

CHAPTER 5

CONCLUSION

5. Conclusion:

In this study, we successfully fabricated the Calcium Alginate beads based implants that could present a potential candidature for controlled drug delivery and tissue engineering application. The bead based implant is a novel structural design wherein each bead could be taken as a single unit of the implant making it more flexible in term of its design and mode of application. The analysis demonstrated that the structure was highly biocompatible, highly hemocompatible and demonstrated good stress relaxation properties. The drug release from the implant could be controlled easily as a function of number of bead layers in implant. However, the applications of the implant need a proper validation in *in vivo* system.

REFERENCES

7. REFERENCES:

1. Wang, M., *Developing bioactive composite materials for tissue replacement.* Biomaterials, 2003. **24**(13): p. 2133-2151.
2. Badylak, S.F., et al., *The use of extracellular matrix as an inductive scaffold for the partial replacement of functional myocardium.* Cell transplantation, 2006. **15**(Supplement 1): p. 29-40.
3. Rezwan, K., et al., *Biodegradable and bioactive porous polymer/inorganic composite scaffolds for bone tissue engineering.* Biomaterials, 2006. **27**(18): p. 3413-3431.
4. Park, S.-N., J.K. Kim, and H. Suh, *Evaluation of antibiotic-loaded collagen-hyaluronic acid matrix as a skin substitute.* Biomaterials, 2004. **25**(17): p. 3689-3698.
5. Kimura, Y., et al., *Adipose tissue engineering based on human preadipocytes combined with gelatin microspheres containing basic fibroblast growth factor.* Biomaterials, 2003. **24**(14): p. 2513-2521.
6. Jiang, Y., et al., *Preparation and characterization of a prolonged and sustained drug delivery system: Linear polyacrylamide in poly (N-isopropylacrylamide)/clay hydrogels.* Journal of Applied Polymer Science, 2012. **125**(S1): p. E148-E156.
7. Zhang, J., S. Xu, and E. Kumacheva, *Polymer microgels: reactors for semiconductor, metal, and magnetic nanoparticles.* Journal of the American Chemical Society, 2004. **126**(25): p. 7908-7914.
8. Paquet, C. and E. Kumacheva, *Nanostructured polymers for photonics.* Materials Today, 2008. **11**(4): p. 48-56.
9. Feyen, M., et al., *Synthesis of structurally stable colloidal composites as magnetically recyclable acid catalysts.* Chemistry of Materials, 2010. **22**(9): p. 2955-2961.
10. Weir, M.D., H.H. Xu, and C.G. Simon, *Strong calcium phosphate cement-chitosan-mesh construct containing cell-encapsulating hydrogel beads for bone tissue engineering.* Journal of Biomedical Materials Research Part A, 2006. **77**(3): p. 487-496.
11. Hutmacher, D.W., *Scaffolds in tissue engineering bone and cartilage.* Biomaterials, 2000. **21**(24): p. 2529-2543.
12. Mandal, B.B. and S.C. Kundu, *Calcium alginate beads embedded in silk fibroin as 3D dual drug releasing scaffolds.* Biomaterials, 2009. **30**(28): p. 5170-5177.
13. Sheridan, M., et al., *Bioabsorbable polymer scaffolds for tissue engineering capable of sustained growth factor delivery.* Journal of Controlled Release, 2000. **64**(1): p. 91-102.
14. George, M. and T.E. Abraham, *Polyionic hydrocolloids for the intestinal delivery of protein drugs: alginate and chitosan—a review.* Journal of Controlled Release, 2006. **114**(1): p. 1-14.
15. Liu, L.-S., et al., *Controlled release of interleukin-2 for tumour immunotherapy using alginate/chitosan porous microspheres.* Journal of Controlled Release, 1997. **43**(1): p. 65-74.
16. Gorodetsky, R., et al., *Fibrin Microbeads (FMB) as Biodegradable Carriers for Culturing Cells and for Accelerating Wound Healing1.* Journal of investigative dermatology, 1999. **112**(6): p. 866-872.
17. Hench, L.L. *Bioactive glasses and glass-ceramics*. in *Materials science forum*. 1998. Trans Tech Publ.
18. Cao, W. and L.L. Hench, *Bioactive materials.* Ceramics international, 1996. **22**(6): p. 493-507.
19. Thevenot, P., W. Hu, and L. Tang, *Surface chemistry influence implant biocompatibility.* Current topics in medicinal chemistry, 2008. **8**(4): p. 270.
20. Rathore, M.H., *The Unique Issues of Outpatient Parenteral Antimicrobial Therapy in Children and Adolescents.* Clinical Infectious Diseases, 2010. **51**(Supplement 2): p. S209-S215.
21. Aimin, C., et al., *Antibiotic loaded chitosan bar: an in vitro, in vivo study of a possible treatment for osteomyelitis.* Clinical Orthopaedics and Related Research, 1999. **366**: p. 239-247.
22. Preissman, H., *Enhanced visibility materials for implantation in hard tissue*, 2001, Google Patents.
23. Meyers, W.E. and T.R. Tice, *Living cells encapsulated in crosslinked protein*, 1989, Google Patents.

24. Schirner, M., et al., *Antiangiogenic chemotherapeutic agents: characterization in comparison to their tumor growth inhibition in human renal cell carcinoma models.* Clinical cancer research, 1998. **4**(5): p. 1331-1336.
25. Wahlig, H., et al., *The release of gentamicin from polymethylmethacrylate beads. An experimental and pharmacokinetic study.* Journal of Bone & Joint Surgery, British Volume, 1978. **60**(2): p. 270-275.
26. Jain, J.P., et al., *Fatty acid based biodegradable polymer.* Polymer Reviews, 2008. **48**(1): p. 156-191.
27. Meyer, J.D., et al., *Preparation and in vitro characterization of gentamycin-impregnated biodegradable beads suitable for treatment of osteomyelitis.* Journal of pharmaceutical sciences, 1998. **87**(9): p. 1149-1154.
28. Wang, L., et al., *Evaluation of sodium alginate for bone marrow cell tissue engineering.* Biomaterials, 2003. **24**(20): p. 3475-3481.
29. Hari, P., T. Chandy, and C.P. Sharma, *Chitosan/calcium–alginate beads for oral delivery of insulin.* Journal of Applied Polymer Science, 1996. **59**(11): p. 1795-1801.
30. Al-Musa, S., D. Abu Fara, and A. Badwan, *Evaluation of parameters involved in preparation and release of drug loaded in crosslinked matrices of alginate.* Journal of Controlled Release, 1999. **57**(3): p. 223-232.
31. Mumper, R.J., et al., *Calcium-alginate beads for the oral delivery of transforming growth factor-< i> β</i>< sub> 1</sub>(TGF-< i> β</i>< sub> 1</sub>): stabilization of TGF-β< sub> 1</sub> by the addition of polyacrylic acid within acid-treated beads.* Journal of Controlled Release, 1994. **30**(3): p. 241-251.
32. Pasparakis, G. and N. Bouropoulos, *Swelling studies and in vitro release of verapamil from calcium alginate and calcium alginate–chitosan beads.* International journal of pharmaceutics, 2006. **323**(1): p. 34-42.
33. Bajpai, S. and S. Sharma, *Investigation of swelling/degradation behaviour of alginate beads crosslinked with Ca< sup> 2+</sup> and Ba< sup> 2+</sup> ions.* Reactive and Functional Polymers, 2004. **59**(2): p. 129-140.
34. Choi, B., et al., *Preparation of alginate beads for floating drug delivery system: effects of CO< sub> 2</sub> gas-forming agents.* International journal of pharmaceutics, 2002. **239**(1): p. 81-91.
35. Shilpa, A., S. Agrawal, and A.R. Ray, *Controlled delivery of drugs from alginate matrix.* Journal of Macromolecular Science, Part C: Polymer Reviews, 2003. **43**(2): p. 187-221.
36. Goswami, S., J. Bajpai, and A. Bajpai, *Calcium alginate nanocarriers as possible vehicles for oral delivery of insulin.* Journal of Experimental Nanoscience, 2012(ahead-of-print): p. 1-20.